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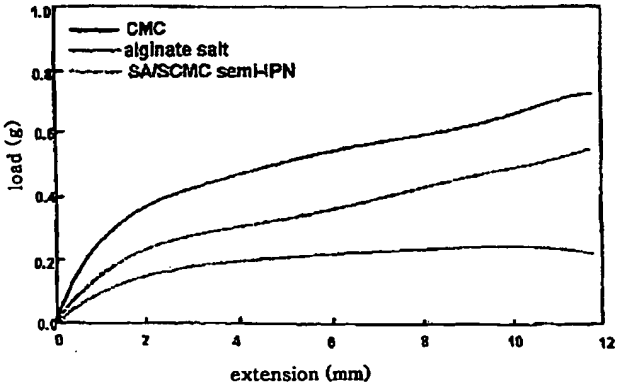
(54) Title: ANTIADHESION BARRIERS CONTAINING WATER-SOLUBLE ALGINATE AND CARBOXYMETHYL CELLULOSE AS MAJOR COMPONENTS AND PREPARATION METHOD THEREOF



(57) Abstract: The present invention relates to an antiadhesion barrier preventive of the formation of adhesions attributable to surgical operation, infection, trauma, etc. and a method for preparing such an antiadhesion barrier. The antiadhesion barrier of the present invention is composed mainly of water-soluble and carboxymethyl cellulose with the alginate crosslinked by calcium ions. The barrier has a semi-interpenetrating network structure as a result of the crosslinking of the alginate with the calcium ions. In addition, to being superb in reattachment and bioreadhesiveness, the antiadhesion barrier of the present invention shows high structural integrity maintenance and retains a certain degree or higher of strength even in a wet state. Also, it lacks blood anticoagulant effects, so that it can be applied for the surgical operations in which the surgical sites are relatively large or bleeding flows profusely. It can effectively prevent the formation of adhesion during injury healing. Further, the antiadhesion barrier of the present invention is easy to handle and useful in surgical operations and secondary operative procedures.

WO 01/05370 A1

ANTIADHESION BARRIERS CONTAINING WATER-SOLUBLE ALGINATE AND CARBOXYMETHYL CELLULOSE AS MAJOR COMPONENTS AND PREPARATION METHOD THEREOF

FIELD OF THE INVENTION

The present invention relates to antiadhesion barriers for preventing a formation of adhesions attributable to surgery, infection, trauma and the like. More particularly, the present invention relates to antiadhesion barriers comprising water-soluble alginate and carboxymethyl cellulose as major components, which have semi-interpenetrating network structures by crosslinking the water-soluble alginate with calcium ion selectively, and a method thereof.

BACKGROUND

Adhesions are indicated that fibrous tissues which are excessively grown between adjacent body tissues during healing of injured tissue resulting from surgery or inflammation, adhere to the adjacent body tissue abnormally. Generally, the adhesions occur at a frequency of 67~93 % after an abdominal operation. Some of them are spontaneously removed, but in most cases, adhesions remain even after healing, thereby causing various complications. After undergoing the abdominal operation, the patient may suffer from the sequalae

due to the adhesions, including intestinal dysfunction, intestinal obstruction, chronic pelvic pain, etc. In particular, the adhesions after the abdominal operation are known to cause infertility (*Eur. J. Surg.* 1997, *Suppl* 577, 32-39).

In contrast to skin, when a defect is made in the peritonium, serofibrinous exudate is secreted around injured site or inflammed site and then fibrin is deposited at an early stage of its healing. This results in fibrin matrices that form fibrinous adhesions to adjoining viscera within 3 hours. Normally, the fibrin matrices are degraded by the action of protease *in vivo*, and absorbed within several days. However, if fibrin matrices are excessively generated over the degradation capacity, they may become organized into fibrinous adhesions through growth of capillaries and fibroblasts and be accumulated around the injured site and adhere to neighboring tissues, resulting in adhesion in the body. In summary, adhesions are generated by a series of fibrinogenesis and fibrinolysis. However the relationship therebetween is not so simple, but intimately associated with healing procedures (*Eur. J. Surg.* 1997, *Suppl* 577, 10-16; *Eur. J. Surg.* 1997, *Suppl* 577, 24-31).

It is known that, when an injury is caused within the body, healing takes for one week (*Surgery* 1995, 117, 663-669). For the adhesion prevention, an adjuvant is used, the surgical site or the injured site to separate from neighboring tissues

during the healing stage. Drugs that inhibit the steps in a series of wound healing could be administered.

Drugs widely used for adhesion prevention include non-steroidal anti-inflammatory drugs, anticoagulants, and fibrinolytics such as tissue-plasminogen activator (t-PA). All except t-PA, are inhibitory against the deposition of fibrin, spontaneously generated during the healing of the injury, so that they have an adverse effect of impairing the healing relative to their antiadhesion effect. The careful consideration should be taken to use such drugs for preventing adhesions *(Eur. J. Surg.*, 1997, *suppl 577*, 32-39; *Fertil. Steril*, 1994, *61*, 219).

Apart from drugs, researchers have recently endeavored to develop antiadhesion barriers, which are capable of preventing surgical sites from adjacent tissues by covering or surrounding the surgical sites. The biocompatible polymers of high molecular weight having terminal carboxy group have been developed as antiadhesion barriers. The antiadhesion barriers are hydrated *in vivo*, separate tissues each other during healing, so that adhesions between wound and normal tissues are not formed. After healing is completed, the antiadhesion barriers are eliminated spontaneously and the affected tissues can be normally functioned.

A variety of biopolymers were developed under this purpose. US patent No. 4,141,973, for example, discloses hyaluronic acid (HA) as an adhesion preventive. However, HA shows

limited antiadhesion efficacy because it is rapidly degraded and absorbed *in vivo*.

Methyl cellulose and its derivatives are known to prevent an adhesion, particularly sodium carboxymethyl cellulose (SCMC) (Fertil. Steril, 1984 Jun., 41:6, 926-928; Fertil. Steril., 1984 Jun., 416, 926-932; Am. J. Obstet. Gynecol., 1986, 155:3, 667-670). However, a solution containing the methyl cellulose or its derivatives is absorbed rapidly, thus it could not exhibit a desired antiadhesion effect.

In order to retard the absorption and/or degradation of such biopolymers *in vivo*, several methods were suggested to reduce their solubilities by intramolecular crosslinking.

European patent No. 507,604 disclosed the use of a carboxy-ended polysaccharide which shows decreased solubility by forming ionic bond with polyvalent metal ions as an adhesion preventive. The polysaccharide has longer residence time in the abdominal cavity, however, the extended period of the residence time does not guarantee antiadhesion efficacy. Rather, the metal ions, if excessively used, may serve as a factor of causing adhesions in the abdominal cavity (*Eur. J. Surg.*, 1997, *Suppl* 577, 32-39). In addition, hydrogel or film were prepared by crosslinking polysccharide with metal ions but was disintegrated easily.

In US patent No. 5,266,326, it is disclosed a method of forming *in situ* barrier with a metal crosslinked alginate hydrogel by injecting a sodium alginate solution and a solution

of metal ions into a surgical site at a time with specialized syringes. This *in situ* gelation method, which allows the synchronous formation of hydrogel by injection, is advantageous in that two fluids are formed into the hard hydrogel *in vivo*, but has lack of tissue adherence. When ligid formulations are injected with the crosslinking agent, they are crosslinked from its surface so that they lose adhesiveness to body tissues and thus, the efficacy of adhesion prevention is reduced. In addition, due to excessive metal ion in the solution, adhesion may occur apart from the wound site in the abdominal cavity. Further more, additional complicated device is required for the injection.

US patent No. 5,318,780 also discloses an *in situ* gelation method for adhesion prevention, in which a film-forming polymer (e.g., hydroxypropyl methyl cellulose (HPMC)) and an ionic polysaccharide are mixed along with metal ions to produce a film, *in vivo*. The mentioned in situ gelation method has several disadvantages for preventing adhesion. By *in situ* gelation, the film may be formed in the body cavity just after the administration of the formulation. However, the adhesion barrier cannot be placed on the desired position of wound for sufficient time interval needed to be cured, not only because film forming polymer cannot show satisfactory adhesiveness to body tissues, but because ionic polysaccharide-multivalent cation complex created after administration, which has no adhesiveness of all. Besides,

once the film is formed on the wound site by the in situ gelation, the formulation does not show any reattachability to body tissues.

Adhesion prevention methods using hyaluronic acid (HA) and carboxymetyl cellulose (CMC) disclosed in US patent No. 5,017,229, 5,527,893 and 5,760,200. According to these inventions, HA and CMC are reacted with EDC (1-ethyl-3(3-dimethylaminopropyl) carbodiimide hydrochloride) to produce polyelectrolyte complexes in which positively charged EDC is electrically bonded to the negatively charged terminal carboxy groups. The compounds of this type, in which positive charges and negative charges coexist, form hydrogel structures which are not easily dissolved nor readily degraded by virtue of their intermolecular ionic bonds. When dried, the hydrogel becomes an antiadhesion film which is highly absorptive while not being readily degraded *in vivo*. EDC, however, has relatively high toxicity, so that requires dialysis process for a long period of time for its removal. Another disadvantage of said film is that great care should be taken in handling and applying it. For instance, lack of softness and strength makes the film highly fragile upon drying. Further, once being applied to wet surfaces of body tissues, they cannot be detached and/or repositioned because they undergo rapid gelation under a hydration condition (*Surg. Clin. Nor. Am.*, 1997, 77:3, 671-688).

US patent No. 5,906,997 discloses a method for making

an antiadhesion composition made of carboxypolysaccharide (CPS) and polyether, whose solubility is controlled by their pH. Membranes prepared by said method are known to exhibit a good adherence to body tissues because they can absorb relatively large amount of water on contact with body tissues. However, as the tissue adherence of the membranes is based merely on hydration, they cannot show adherence after being saturated with water. Thus, there remains a need for an improved method which allows membranes to maintain high adherence to body tissue after saturation with water.

Bioadhesion means the adhesion of polymers, biopolymers and/or body tissues to other body tissues. The bioadhesion is generally observed (*J. Controlled Release* 1985, *2*, 257). In dental surgery and orthopedic surgery fields, bioadhesion is extensively utilized to adhere adjuvants to body tissues (*Adhesion in Biological Systems* Academic Press N.Y. 1970). There are three major reasons in adhesion formation between biopolymers and body tissues; chemical bond, Van der Waals force and the similar, and surface interaction between the polymers and body tissues. A variety of hypotheses are suggested in order to explain such adhesion phenomena (*Bioadhesive Drug Delivery Systems* CRC Press 1990). Primary adhesion between thin films and body tissues takes place therebetween through hydration or hydrogen bonding, and then the adhesive force reaches an equilibrium, and finally terminal carboxy groups infiltrate through the tissues of opposing sides

(*Macromolecules* 1980, 13, 880). In this regard, the adhesion is affected by two properties, tackiness and adherence: the former is related to the adhesion achieved by the hydration of the early stage while the latter dominates the adhesion which is accomplished by direct intermolecular bonds after completion of the hydration. The adhesion of biopolymers or synthetic polymers to body tissues is also conducted in two stages which are driven dominantly by the tackiness according to the hydration and the adherence by intermolecular bonds, respectively (*J. Pharm. Sci.* 1982, *71*, 975; *J. Pharm. Pharmacol*, 1982, *34*, 70).

There are several factors to determine the adherence of polymers themselves to body tissues, which appears after the tackiness stage. The most generally accepted one is a diffusion theory. The terminal carboxy groups on the body tissues and those on the biopolymers which adhere to the body tissues, are diffused into and entangled with each other. (*J. Controlled Release* 1985, 2, 257).

As described above, the adhesion of hydrophilic biopolymers to body tissues in an early stage, is affected predominantly by the hydration irrespective of compositions and structures. However, in order for the polymers and tissues to retain adherence even after they are saturated with water, the polymers themselves must have terminal carboxy groups that can be bonded to the body tissues. In the case of gel formulations, since they are already saturated with water,

the adherence of the polymers themselves is more important.

By the way, antiadhesion barriers as mentioned above may be formulated into solutions, gels and films.

Because serious damage to the skin or tissues may cause adhesion between the tissues, recent operative techniques have been directed to minimizing the injury and damage of the skin and the tissues. This change in operation techniques also gives an additional effect of restraining the adhesion between tissues (*Hepato-Gastroenterol,* 1991, 38, 283).

In response to the change of operative techniques, the recent development tendency of antiadhesion barriers has been directed to solution or gel formulations (*The Adhesion Prevention Opportunity,* Report from MDI, 1998).

Solution formulations are administered when they should be used in a large quantity after surgical operations tsuch as surgery for the abdominal or the pelvic cavity. However, they have not been employed actively on account of the psychological burden of using a large quantity of foreign materials although they are almost excreted out within two or three days *(Eur. J. Surg.* 1997, *Suppl* 577, 32-39).

On the contrary, gel formulations have recently come into the spotlight on account that application of even a small quantity to an injured site can act as an adjuvant for effectively preventing adhesion. So far, a gel formulation limitedly applicable for the surgical operations on the lumbar and tendons has been developed (U.S. Pat. No. 5,605,938).

The gel formulation, characterized in that dextran sulfate is used as an active material with a protein binder, is based on the fact that dextran sulfate is able to prevent the approach of glial cells, which are involved in the production of fibrous tissues. The gel formulation has advantages of being convenient in its use and preventing the adhesion of not only intended, but unintended sites. This technique is however limited in its use. It cannot be applied for surgical operations where surgical sites are relatively large or bleeding profusely because dextran sulfate inhibits against blood coagulation. The gel formation can be used only for delicate surgical operations such as operations on the lumbar and tendons.

As for film formulations for antiadhesion barrier, they are useful if it can be detached just after application to surgical sites and then can be applied again because the detachment and the reattachment frequently occurs in practice. Therefore, film formulations that have good primary adherence as well as excellent secondary adherence are preferred.

The intensive and thorough research on antiadhesion barriers repeated by the present inventors aiming to realize desired tissue adhesiveness and structural integrity. They found that a semi-interpenetrating network structure, comprising water-soluble alginate and carboxymethyl cellulose as major components, in which only the alginate is crosslinked with calcium ions, is highly preventive of post-operative adhesions in terms of bioadhesiveness and structural

integrity.

SUMMARY OF THE INVENTION

It is an object of the present invention to overcome the above-mentioned problems encountered in prior arts and to provide antiadhesion barriers which effectively inhibit or prevent the formation of *de novo* adhesions during or after surgical operations. The antiadhesion barrier of the present invention also can be used to prevent the re-occurrence of adhesions upon the secondary operations that are conducted to remove the adhesions formed upon the primary operations.

It is another object of the present invention to provide antiadhesion barriers that are inexpensive and can maintain their structural integrity, which is very important in early stages of healing process of injury.

It is a further object of the present invention to provide biocompatible antiadhesion barriers, which are flexible to easily handle in use and have enough strength not to be easily tore.

It is still a further object of the present invention to provide antiadhesion barriers, which retain strength up to a certain level even in a wet state so they can be re-applied to injured sites after being detached from body tissues.

It is still another object of the present invention to provide antiadhesion barriers which maintain structural

integrity within the body during a period of healing and be absorbed and/or eliminated thereafter.

It is yet another object of the present invention to provide antiadhesion barriers which can incorporate appropriate drugs and release them locally in a sustained manner during a period of healing.

BRIEF DESCRIPTION OF THE DRAWING

Fig. 1 is a graph showing that hydration behavior of a semi-IPN structural formulation is more similar to that of a formulation with carboxymethyl cellulose than that of a formulation with alginate.

DETAILED DESCRIPTION OF THE INVENTION

In order to obtain above-mentioned objects, the present invention provides antiadhesion barriers comprising water-soluble alginate and carboxymethyl cellulose as major components, wherein the water-soluble alginate is selectively crosslinked with calcium ion. The antiadhesion barriers are characterized in that they have semi-IPN structures formed by crosslinking of the alginate with calcium ion.

Further, to provide antiadhesion barriers with desired adhesiveness, structural integrity and reattachment, the calcium ion must be present at an appropriate amount. An

excessive calcium ion is crosslinked the carboxymethyl cellulose as well as the alginate and causes the antiadhesion barriers to slip away from surgical sites because the antiadhesion barriers are unable to adhere to body tissues after being saturated with water. In addition, an excessive amount of metal ion may cause the formation of *de novo* adhesions. On the other hand, if calcium ion is insufficient, the resulting antiadhesion barriers are not sufficiently crosslinked and cannot be sustained in the body during the period for injury healing. In this regards, a weight ratio of water-soluble alginates to calcium ions having antiadhesion efficacy ranges from 1:0.05 to 1:0.2. A semi-IPN structure in which sodium alginates are selectively crosslinked with calcium ions, while carboxymethyl celluloses are not crosslinked is formed in the range of the above-mentioned weight ratio of water-soluble alginates to calcium ions.

In accordance with the present invention, weight ratio between sodium carboxymethyl celluloses and sodium alginates is determined to optimize the performance of the antiadhesion barriers. The excess of alginate can maintain the structural integrity of the antiadhesion barriers for longer time, however it may reduce adhesiveness of the antiadhesion barriers to body tissues. Adversely, excess of carboxymethyl cellulose may not be sustained in the body during the period for injury healing because the polysaccharides are degraded and absorbed rapidly. The alginates are preferably used at an amount of

90~10 wt% and more preferably 50~10 wt%. Conversely, the carboxymethyl celluloses are preferably used at an amount of 90~10 wt% and more preferably 90~50 wt%.

Antiadhesion barriers of the present invention can be prepared in a gel or film form.

In addition, the present invention provides a method for preparing antiadhesion barriers which comprise the steps of; 1) dissolving a mixed powder of alginates and carboxymethyl cellulose in water or mixing an alginate solution and a carboxymethyl cellolose solution to produce a solution; and 2) adding a calcium ion solution to the solution while slowly stirring to give a gel solution.

Further, the present invention provides antiadhesion barriers which can incorporate drugs and release the drugs locally during injury healing. The available drugs include non-steroidal anti-inflammatory drugs, anticoagulants, protein hydrolyzing agents, and tissue growth factors.

Before giving details of the invention, the terms as used herein are defined as follows:

The term "water-soluble alginate" means a metal salt of alginic acid, a polysaccharide consisting of mannuronic acids and guluronic acids, which is soluble in water. The term "sodium alginate (SA)" means a water-soluble alginate wherein the metal ion is sodium. The term "alginate" means a polysaccharide after the metal ion is dissociated from

the water-soluble alginate upon dissolution in water.

The term "sodium carboxymethyl cellulose (SCMC)" means a sodium salt of a polymer consisting of repeating cellobiose units which are linked by 1,4-glycosidic linkages and some of their hydroxy groups are substituted with carboxymethyl groups. The term "carboxymethyl cellulose (CMC)" means a polymer after the sodium ion is dissociated from the SCMC upon dissolution in water.

The term "bioadhesive" means being able to adhere to body tissues. The term "reattachment" means being able to re-adhere to body tissue after detachment.

The term "structural integrity" means remaining intact at the applied tissue sites during wound healing.

The term "hydrogel" means a three-dimensional network of a hydrophilic polymer which retains a large quantity of water. The term "semi-interpenetrating network (semi-IPN)" means a network structure of two polymers in which one is selectively crosslinked without affecting the other. The term "interpenetrating network (IPN)" means a network structure of two polymers in which both are crosslinked respectively, but without affecting each other.

The present invention provides a composition which is prepared by mixing a water-soluble alginate solution with a SCMC solution and selectively crosslinking the alginate with a calcium ion solution. Additionally, the present

invention prevents or inhibits the formation of adhesions between injured tissues resulting from surgical operation and adjacent tissues, whether injured or not. The antiadhesion barriers prepared by the method of the present invention are easy to handle and superior in bioadhesiveness and reattachability with excellent structural integrity in the abdominal cavity.

The properties of antiadhesion barriers at which the present invention aims, including superb bioadhesiveness, reattachment and structural integrity within the body, can be obtained by closely controlling a mixing ratio of the calcium solution, the SCMC solution and the water-soluble alginate.

In accordance with the present invention, the alginate is selectively and strongly crosslinked by forming ionic bonds with calcium ions and thus, enabled to successfully perform the antiadhesion function while the other component, that is, the non-crosslinked CMC is responsible for adhesiveness to body tissues. With this structure, the antiadhesion barrier of the present invention can exhibit maximum degrees of bioadhesiveness, reattachment, and structural integrity within the body. The antiadhesion barriers of the present invention can be formulated into gel and film, both. The film-type antiadhesion barriers of the present invention have advantages over conventional polysaccharide films in that they are not readily torn and

can be reattachable.

Sodium alginate (SA) is inexpensive and is known to be ionically crosslinked with multivalent metal ions, especially calcium ion, resulting in a strong structure of hydrogel. In the hydrogel structure, so-called egg-box model, calcium ion is present in the crevice between two opposing terminal carboxy groups of alginate polymers, like egg in the egg box (*Biodegradable Hydrogels for Drug Derilvery*, 1993, p. 116). The bonding force between calcium ions and alginate is so powerful that the calcium-alginate gel structure is rarely disintegrated unless magnesium ion or sodium ion is present at a high concentration or chelating agents which have strong bonding force to calcium ion are present. Accordingly, the alginate which is crosslinked with metal ions, show high structural integrity in the body and is allowed to play a role as a barrier.

To obtain the antiadhesion barrier properties at which the present invention aims, the inventors paid attention to the fact that, when a solution of water-soluble alginate and SCMC in water is treated with calcium ions, the alginate is selectively crosslinked, resulting in a network structure which can remain intact with superb antiadhesion activity and adhere to body tissues during the period for injury healing. The crosslinking between the alginate and the calcium ion does not result from a chemical reaction, but from a physical interaction, namely an ionic bond with metal ion, so that

a certain adverse effect does not occur in the body.

SA, a biopolymer used in the present invention, is crosslinked in the presence of calcium ions to form a rigid hydrogel of the egg-box structure. A film prepared by drying said hydrogel is similar in initial tackiness in a dry state to the film made of CMC only, and a semi-IPN film or a IPN film made of CMC and alginate, but in a wet state, far inferior in the reattachability as well as the bioadhesiveness as shown in Table 3, which will be described later in Examples. The phenomenon can be explained as follows; a hydration of the dry film is a major driving force of initial tackiness, but when the hydration reaches an equilibrium, the film cannot exhibit bioadhesiveness because there are few free carboxy groups that can adhere to body tissues and carboxy groups take part in the crosslinking instead.

In order to overcome the reduction of bioadhesiveness, CMC is introduced in the present invention. CMC is a kind of cellulose derivatives made by introducing carboxy end groups with various degrees of substitution. It is known to be biocompatible, inexpensive and easily obtainable. In addition, CMC can be readily molded into a thin film by casting and drying an aqueous CMC solution. The film has high water uptake capacity and becomes tacky upon hydration. Especially, it has high adhesiveness to body tissues by virtue of the diffusion of the end carboxy groups of CMC as mentioned above.

In contrast to alginate, which forms very strong hydrogel

structure on contact with calcium ions, strong hydrogel structure of CMC can be formed by the calcium ion solution of high concentration (*Biodegradable Hydrogels for Drug Delivery*, 1993, p 119). By controlling concentration of calcium ion appropriately, crosslinking can be formed between alginate and calcium ion, without crosslinking CMC. A structure composed of two polymeric components in which one of the two polymeric components is selectively crosslinked while the other is not affected, is called a semi-interpenetrating network (semi-IPN). On the other hand, a structure in which the two polymeric components both are crosslinked without affecting each other, is called an interpenetrating network (IPN).

As explained, the addition of an appropriate amount and concentration of calcium ions in an aqueous solution of SA and SCMC in water can produce a semi-IPN in which intact CMC is interposed between the network structures of the rigid hydrogel formed as a result of the crosslinking of the alginate with the calcium ion.

The concentration of calcium ion is important to determine the structure of hydrogels. When an aqueous solution of SA and SCMC is formulated into a film with a high concentration of calcium ion, the CMC is also crosslinked to form an IPN structure. In the initial stages of surgical operation, the IPN structure can exert bioadhesiveness on surgical sites by virtue of the hydration of the film itself. However, once

the IPN structure film is saturated with water, it cannot adhere to the body tissue, but slips away from the administered site after operation. In addition, the film cannot be completely reabsorbed and eliminated from the body owing to its high structural integrity. What is worse, excessively added metal ion, may cause the formation of *de novo* adhesions. On the other hand, if calcium ions are insufficient, the resulting formulation is not sufficiently crosslinked and cannot be maintained in the body for the period of time necessary for injury healing because the polysaccharide is degraded and absorbed rapidly. In other words, the formulation cannot play a sufficient role as an antiadhesion barrier.

Therefore, by precisely regulating the amount of calcium ions, there can be obtained an antiadhesion barrier which is easy to handle and shows optimal structural integrity with excellent bioadhesiveness and reattachability. In case of the semi-IPN of the antiadhesion barrier comprising SA, SCMC and calcium ion, the weight ratio of SA to calcium ion ranges from 1:0.05 to 1:0.2.

Not only the amount of calcium, but the weight ratio between SCMC and SA is also important factor in determining the bioadhesiveness, reattachment and structural integrity of the antiadhesion barrier.

For example, if insufficient SCMC is used relative to SA, the resulting formulations maintain their structural integrity for a long period of time, but are deficient in

bioadhesiveness. On the other hand, if excess SCMC is used, the resulting formulations are lack of structural integrity, so that they are degraded and absorbed rapidly. To achieve sufficient bioadhesiveness and structural integrity at which the present invention aims, SCMC is within the range of 90~10 wt% while SA is within the range of 90~10 wt% in a mixture of SCMC and SA. Preferably, SCMC ranges from 50 to 90 wt% and SA ranges from 10 to 50 wt%.

The gel and film formulations of the antiadhesion barrier prepared by the present invention were assayed *in vitro* and *ex vivo* for the adhesiveness to body tissue. The term "*in vitro* adhesiveness assay" as used herein means an adhesiveness test method which does not utilize body tissues, but use a solution of simulating biological condition. The term "*ex vivo* adhesiveness assay" as used herein means an adhesiveness test method which is conducted with a part of a body tissue, but not within the body when the test cannot be conducted directly within the body. *(Bioadhesive Drug Delivery Systems* CRC Press 1990).

The gel formulations prepared according to the present invention were assayed *in vitro* for bioadhesiveness. The assay results demonstrate that the maximum adhesive strength of the semi-IPN structures according to the present invention is between that of the alginate gel and that of the CMC gel. Crosslinked alginate-Ca^{++} and IPN formulations have very low adhesive strength (Table 1).

Since the tackiness resulting from the initial hydration does not impart the adhesive strength of a gel formulation, the adhesive strength of gel materials themselves plays an important role in determining the *in vitro* adhesiveness of the gel formulation. Because both alginate and CMC have bioadhesiveness respectively and do not have particular mutual interactions, a formulation prepared by mixing the two biopolymers simply, exhibits a bioadhesiveness value which is the arithmetic mean of the bioadhesiveness values of the two biopolymers. But alginate-Ca^{2+} formulation cannot have the adhesiveness to body tissues because most of the carboxy groups in alginate are participating in crosslinking with calcium ions. This is also true of the IPN formulations in which each of the two biopolymers is crosslinked respectively.

As for the formulations, composed mainly of water-soluble alginate and SCMC, in which only the alginate is crosslinked with calcium ion, they show bioadhesiveness which comes from CMC alone because the alginate loses its adhesiveness after being crosslinked with calcium ion. SA and SCMC each show different adhesiveness behaviors. The gel formulations of the present invention and SCMC have similar adhesiveness behaviors because only the alginate component is selectively crosslinked with calcium ions in the gel formulations.(see Fig. 1).

Owing to the lack of anticoagulative effect, which is the characteristics of anticoagulant such as dextran sulfate,

the gel formulations of the semi-IPN structure according to the present invention can be used in the surgical operation which leaves relatively large surgical sites. In addition, the formulations themselves have adhesiveness to body tissues to prevent adhesion effectively.

In practical operation procedures, it often occurs that film-type antiadhesion barriers are detached from the surgical sites immediately after operation and then re-attached. (*The market for antiadhesion products*, Pieter Halter, Medical Data International, *Mar.* 2 1996). For this reason, *ex vivo* assay of the bioadhesiveness in a dry state and the reattachment were examined(see Table 3). As previously mentioned, the tackiness of the film formulation in a dry state mainly attributes its hydration, while the adhesive strength of the film, saturated with water, is mainly dependent on the adhesiveness of the film materials themselves to body tissues. In other words, different formulations, although similar in the adhesive force of a dry state, show different adhesive forces when being in a hydrated state.

As shown in Table 3, the initial tackyness of a dried film with a semi-IPN structure is similar to that of a film having an alginate-Ca^{2+} structure or a film consisting of SCMC alone and slightly lower than that of a film having an IPN structure. As explained above, since the tackiness of the film formulations in a dry state is highly achieved by the hydration, polysaccharide films with a certain level

of hydration rates show similar tackiness, regardless of their composition. In reattachment, however, a film having a semi-IPN structure has much larger value than other structures because, while the alginate-Ca^{2+} structure maintain the physical integrity of the film, the terminal caboxy groups of CMC exhibit the adhesiveness to the body tissues. Because of excellent reattachability of the present invention, surgeons easily perform the secondary surgical operation as well as the primary procedure.

The hydration behaviors of formulations in the present invention are shown in Table 2. As apparent from Table 2, approximately two minutes was sufficient to complete the hydration of all the formulations. Based on these data, formulations were hydrated for two minutes and measured for the adhesive strength in a wet state. The results are given in Table 3. Table 3 demonstrates that, in a wet state, a film formulation of a semi-IPN structure of the present invention has a similar adhesive strength to that of SCMC formulation or SA-SCMC mixed formulation, but more higher than that of an alginate-Ca^{2+} or an IPN structure. Therefore, the film formulation prepared by the method of the present invention retains excellent bioadhesiveness even after being hydrated sufficiently.

In summary, the data obtained through the above two adhesiveness tests shows that the formulations of semi-IPN structures not only have sufficient initial adhesiveness

to conduct a primary surgical operation but also show excellent reattachment and further, retain adhesiveness to body tissues even after being completely hydrated.

Strength and elongation are major indices of the physical properties of films. While the strength is closely related to the solidity of films, the elongation exhibits flexibility. In the present invention, the strength and the elongation of the film in a dry state were examined in order to establish a measure of convenience on the first use and in a wet state in order to determine the extent of ease for a secondary operation procedure.

Antiadhesion barriers using conventional polysaccharide films are very inconvenient to handle in use because they are highly brittle. In addition, the conventional antiadhesion barriers are economically disadvantageous in that a number of films should be utilized at a wound site because they are scarcely detached once being attached to a surgical site. However, the films prepared by crosslinking alginate with calcium ions have advantages over other polysaccharide films in terms of both flexibility and strength.

As shown in Table 4, significant differences cannot be found between the strengths of semi-IPN structural films and simply mixed formulations in a dry state. In a wet state, however, film formulations having semi-IPN structures are far superior in strength to other formulations, except film formulations having IPN structures. The reason is that, in

contrast to the conventional formulations easily dissolved in water, the film formulations of semi-IPN structures are more resistant to water due to the crosslinking with calcium ions. Exceptionally, high strength in a wet state in accordance with the present invention makes it possible to overcome the inconvenience on surgical use of conventional polysaccharide film formulations.

The data obtained above demonstrates that the gel and film formulations having semi-IPN structures, which are prepared by mixing water-soluble alginate and SCMC at an appropriate amount and selectively crosslinking only the water-soluble alginate with calcium ions, can function as antiadhesion barriers which have superior bioadhesiveness and reattachability and retain structural integrity for a period of time for injury healing.

Antiadhesion barriers can incorporate drugs and can deliver the drugs to the surgical site in a sustained manner during a period of injury healing. Incorporation of drugs into the barriers may be described in detail by US patent No. 5,578,305. The incorporation may be conducted during the preparation of the formulations. Any drug, if it is compatible with the formulations of the present invention may be used; antithrombogenic agents such as heparin or t-PA, anti-inflammatory drugs such as aspirin or ibuprofen, hormones, analgesics, anesthetics, or others.

To evaluate the antiadhesion efficacy of the compositions

prepared according to the present invention, various formulations were applied to animals. As animal models for testing adhesion formation, rats were selected with reference to *Surgery* 1995, *117*, 663-669. Compared to conventional antiadhesion barriers, the antiadhesion barriers of the present invention were proven to be excellent in the inhibition against the formation of adhesion.

EXAMPLES

Practical and presently preferred embodiments of the present invention are illustrative as shown in the following Examples.

However, it will be appreciated that those skilled in the art, on consideration of this disclosure, may make modifications and improvements within the spirit and scope of the present invention.

Example 1: Preparation of Gel and Film Formulations Having Semi-IPN Structures

1-1: Formation of Semi-IPN Structure Gel from CMC and Alginate Powders

With 8 g of CMC powder, 2 g of alginate were mixed,

and the mixture was slowly added for 5 min in deionized water while stirring at 400 rpm by means of a mechanical stirrer. When the solution became viscous as the powders were dissolved, the stirring speed was reduced to 120~150 rpm, at which stirring was further performed for 4 hours to give a completely homogeneous solution. While being stirred at 300~350 rpm, the homogeneous solution was added with calcium ions at an amount of 0.1 times as much as the weight of SA. In order to form a uniform semi-IPN structure, calcium ions were slowly added for 10~15 hours.

1-2: Formation of Semi-IPN Structural Gel From SCMC and SA Solutions

2 wt% solution of SA in deionized water was mixed to 2 wt% solution of SCMC in deionized water, followed by mechanical stirring at 120~150 rpm to give a homogeneous solution. While being stirred at 300~350 rpm, the SA-SCMC mixture was added with calcium ions at an amount of 0.1 times as much as the weight of SA. The solution of calcium ions was slowly added for 10~15 in order to afford a uniform semi-IPN structure.

1-3: Preparation of Semi-IPN Structure Film

The gel solutions prepared in (1-1) and (1-2) were molded

into films which were then completely dried at 35~40℃ at an ordinary pressure.

Comparative Example 1: Preparation of Film from SA Solution

To deionized water was added SA powder, followed by mechanically stirring at 350~400 rpm to give a solution. This was molded into a thin SA film which was dried under the same conditions as in Example 1-3.

Comparative Example 2: Preparation of Film from SCMC Solution

The same procedure as in Comparative Example 1 was repeated, except using SCMC powder instead of SA powder, to prepare an SCMC film.

Comparative Example 3: Preparation of Film from SA-Ca^{2+} Solution

The SA solution obtained in Comparative Example 1 was molded to a thin film which was then immersed in a 0.2~5.0% calcium solution for 1~30 min to make an SA-Ca^{2+} structure. Drying the structure in the same manner as in Example 1-3 afforded a film.

Comparative Example 4: Preparation of Film from SCMC-Al^{3+}

Solution

The SA solution obtained in Comparative Example 2 was molded to a thin film which was then immersed in a 0.2~5.0% aluminum solution for 1~30 min to make an SCMC-Al^{2+} structure. Drying the structure in the same manner as in Example 1-3 afforded a film.

Comparative Example 5: Preparation of Film from SA-SCMC Mixed Solution

The simply mixed SA-SCMC solution prepared in Example 1-2 was molded into a thin film which was then dried under the same conditions as in Example 1-3.

Comparative Example 6: Preparation of IPN Structure Film

The simply mixed SA-SCMC solution prepared in Example 1-3 was molded into a thin film. The film was then immersed in a 5~15% calcium ion solution for 2~12 hours to allow CMC as well as alginate to be completely crosslinked to afford an IPN structure. Successively drying was performed under the same conditions as in Example 1-3 to give an IPN structural film.

Example 2: Preparation of Semi-IPN Structure Film

To investigate the effect of calcium concentration on the performance of antiadhesion barriers, various formulations containing different calcium concentration were performed. A gel formulation was prepared in a similar manner to that of Example 1, except the fact that calcium ions were added at an amount of 0.2 times as much as the weight of SA. From the gel formulation, a film was obtained in the same manner as in Example 1-3.

Example 3: Preparation of Semi-IPN Structure Film

The same procedure as in Example 2 was repeated, except for using calcium ions at an amount of 0.05 times as much as SA, to give a film.

Example 4: Preparation of Semi-IPN Structure Film

In order to determine the influence of SA-SCMC ratios on the performance of antiadhesion barriers, various semi-IPN structure film formulations were prepared which were different from one to another in the ratio between SA and SCMC. A semi-IPN structure formulation was obtained in a similar manner to that of Example 1, except that a solution containing 9 g of CMC and 1 g of alginate was added with calcium ions at an amount of 0.1 times as much as the weight of SA. The

formulation was molded into a thin film which was then completely dried at 35~40°C at an ordinary pressure.

Example 5: Preparation of Semi-IPN Structure Film

A film formulation was prepared in a similar manner to that of Example 4, except that 5 g of CMC and 5 g of alginate were used.

Example 7: Preparation of Semi-IPN Structure Film

A film formulation was prepared in a similar manner to that of Example 4, except that 2 g of CMC and 8 g of alginate were used.

Experimental Example 1: Structural Integrity Assay

For *in vitro* structural integrity tests, films prepared under various conditions were cut into a specified size and allowed to shake at 60 rpm in a phosphate buffered saline (PBS) pH 7.4 at 37 ℃. The degradation of the films in PBS was evaluated by measuring the time needed for the films to lose their film shapes when being observed with the naked eye. Gel formulations were also tested under the same conditions for *in vitro* degradation.

For *in vivo* structural integrity tests, various

formulations of antiadhesion barrier were applied for female SD rats with a body weight of 200~250 g, according to an animal model experiment disclosed in *Surgery* 1995, *117*, 663-669. Every 1st, 2nd, 3rd, 4th, 5th, 6th, 7th, 11th, and 14th day after a surgical operation, three rats were randomly selected from each animal group of 27 rats and underwent laparotomy to observe the shapes of the antiadhesion barriers applied to the surgical sites under the naked eye.

As for the semi-IPN structure film formulation of Example 1, it was observed to retain its film shape for 12~17 days, to start to lose the shape at the 16~17th day, and to dissolve completely after 20 days, as a result of the *in vitro* assay.

Under the *in vivo* conditions, the semi-IPN structure film formulation of Example 1 remained intact at the surgical site until 7 days after an operative procedure. Even at 14 days after the operation, the film was completely absorbed, leaving a little residue.

Examples 2 and 3, semi-IPN structure film formulations with different calcium concentration, were also tested for the *in vitro* and *in vivo* structural integrity test.

The film formulation of Example 2 maintained its structural integrity longer than that of Example 3 under the *in vitro* conditions. The film formulation of Example 2 maintained its structural integrity until the 28~29th day after the immersion in PBS solution, and then started to lose its shape slowly, disappeared after about 40 days. In

contrast, the film formulation of Example 3 sustained its shape for 7~8 days and since then, its shape was started to collapse. No trace could be found after 13 days.

Under the *in vivo* conditions, the film of Example 2 was observed to maintain its shape at the surgical site until the 7th day after the operation. Even after 14 days, a relatively large portion of the film was left, demonstrating that its degradation rate in a body is slower than that of the film formulation of Example 1. In contrast, the film formulation of Example 3 was degraded faster than that of Example 1. On the third day after application, the film formulation of Example 3 started to lose its shape and after 7 days, no trace could be recognized.

Experimental Example 2: *In vitro* Adhesiveness Assay of Gel Formulation

Cover glasses were immersed in the semi-IPN structure gel obtained in Example 1 and in the gels obtained in Comparative Examples 1 to 6, and then the glasses coated with the gels were dried in the air. The cover glasses were dipped to a depth of 10 mm into 5 wt% mucin suspension. The forces required to pull the cover glasses at a speed of 0.1~2.0 mm/min, were measured to evaluate the adhesiveness of the gel formulations. The instrument used in this example was INSTRON 4465 with a load cell of 250 g. Results of the *in vitro* adhesiveness

experiment are given in Table 1, below.

<TABLE 1> Maximum Adhesive Strength of Gel Formulations

Nos. Of Examples	Max. Load(mg)
C. Example 1	401±53
C. Example 2	754±45
C. Example 3	42±5
C. Example 4	634±55
C. Example 5	29±3
Example 1	611±37
Example 2	540±51
Example 3	619±29
Example 4	627±36
Example 5	548±27
Example 6	505±34

With no influence of the tackiness resulting from the initial hydration, the *in vitro* adhesive strength of gel formulations is determined critically by the adhesive strength that materials themselves have. Therefore, all the formulations of SA, SCMC and SA-SCMC, showed appropriate levels of adhesive strength while SA-Ca^{2+} and IPN gels in which terminal carboxy groups were crosslinked with calcium ions, had very low adhesive strengths. On the other hand, the semi-IPN formulations of the present invention showed similar adhesiveness levels to those of the simply mixed SA-SCMC formulations.

Fig. 1 showed the trends of adhesive strengths of several formulations, semi-IPN gel, SCMC gel, SA gel, which were

measured by pulling cover glasses coated with said formulations from mucin suspension. As seen, the SCMC gel had a gradually increasing curve of a load during the pulling of the cover glass. For the SA gel, the load was increased to a certain degree at early stages of the pulling and then, kept almost constant. The semi-IPN formulation according to the present invention showed a similar adhesive strength behavior to that of the SCMC gel. This similarity could be attributed to the fact that the adhesiveness of the semi-IPN formulation came almost exclusively from CMC because alginate showed little adhesiveness due to the crosslinking with calcium ions. Therefore, the semi-IPN structures prepared according to the present invention retained excellent structure integrities by virtue of the crosslinking of alginate with calcium ion as well as kept the adhesiveness attributable to the terminal carboxy groups of CMC.

Meanwhile, in order to investigate the effect of calcium ion concentrations on the adhesiveness, the semi-IPN gel formulations of Examples 2 and 3 were subjected to the *in vitro* adhesive strength test as described above.

The results were given in Table 1. As compared with that of the gel of Example 1, in vitro adhesiveness of the gel of Example 2 was a little low. It was due to the fact that calcium ion, if presents at a high concentration, can bind to some CMC as well as to alginate, so that the terminal carboxy groups responsible for the bioadhesiveness were

reduced. In the gel of Example 3, on the other hand, the CMC did not form crosslinks with calcium ion to play a critical role in the adhesiveness and some of the alginate not crosslinked with calcium ion also contributed to the adhesiveness. Accordingly, similar adhesiveness behaviors were observed between the gels of Example 1 and 3.

Due to investigate the effect of the ratio between SA and SCMC on the adhesiveness, the semi-IPN gels of Examples 4, 5 and 6 were subjected to the *in vitro* adhesiveness test.

Table 1 showed the results. As recognized from Table 1, the adhesive strength is decreased with the decreasing of the proportion of SCMC. In case of gel formulations, the bioadhesiveness of the polymers themselves were most responsible for their adhesive strengths. Hence, the higher is the ratio of SCMC responsible for the bioadhesiveness, the better is bioadhesiveness of the gel. Correspondingly, higher ratios of SA, which forms crosslinks with calcium ion, have the less bioadhesiveness of the formulations.

Experimental Example 3: Measurement of the Degree of Hydration of Film Formulations

The films formed from various formulations were cut into a predetermined size and weighed. In a 50 ml vial filled with distilled water were soaked the film pieces and, after a predetermined period of time, the film pieces were drawn

out from the vials and weighed to evaluate their water uptakes. Extent of hydration was represented by degree of swelling (%S) calculated according to the following equation:

$$\%S = \frac{wet\ film\ mass - dry\ film\ mass}{dry\ film\ mass} x100(\%)$$

The degrees of swelling of several formulations were given in Table 2, below.

<TABLE 2> Degrees of Swelling of Film Formulations

Hydration Times (sec)	Degrees of Swelling (%)		
	Example 1	C. Example 5	C. Example 6
0	0	0	0
5	417.0	801.6	99.21
10	535.6	813.1	149.1
20	715.9	1051	154.9
30	740.2	1825	154.8
60	1176	2080	231.8
90	1659	3082	300.7
120	3046	-[a]	304.1
150	3054	-[a]	-[b]

[a] impossible to weigh the film owing to decomposition

[b] hydration equilibrium state

The simply mixed SA-SCMC formulation could not be measured virtually for water uptake because it was dissolved as soon as the measurement was conducted. In addition, the formulation based on the SA-SCMC solution of Comparative Example 5 and the IPN formulation of Comparative Example 6 reached hydration equilibrium states after one minute and thus, it was meaningless

to further measure the degrees of swelling. In contrast, because the film of semi-IPN formulation of the Example 1 absorbed water at a relatively slow rate, the time taken to reach the hydration equilibrium state was about 2 min. In the equilibrium state, the semi-IPN formulation showed a degree of swelling of about 3,000 %.

Experimental Example 4: *Ex vivo* Adhesiveness Assay of Film Formulation

After being etherized, female SD rats with a body weight of 200~250 g underwent laparotomy, and the abdominal walls were excised from them and washed with 4℃ physiological saline solution to remove impurities sticking to the abdominal walls. For freshness, they were immersed in 4℃ physiological saline solution to the time just before use in experiments. The excised tissues should be used within 1 hour after excision; otherwise, they were all discarded.

Films prepared from various formulations were cut into a proper size and fixed to the bottom surface of a stainless steel which weighed 10.0 g with a bottom area of 100 mm^2 (10x10 mm). The films attached to the weight were placed on the excised tissues for 2 min, then the weight was pulled at a speed of 0.1~2.0 mm/min to measure the separating forces between the film and the tissue of abdominal walls.

For the reattachment, the films used in the former

experiments were placed on the abdominal wall tissues again and tested in the same manner as in the above. The reattachment means to what extent the adhesive strength of a dry film is maintained in a wet state, and can be calculated as follows:

$$\%\ Reattachment = \frac{2nd\ adhesive\ strength}{dry\ adhesive\ strength} \times 100\ (\%)$$

The following test was conducted to measure the adhesive strength when film or gel formulations were completely hydrated. First, films prepared from various formulations were soaked in physiological saline solution for 2 min, cut into a proper size and fixed to the bottom surface of a stainless steel weight which weighed 10.0 g with a bottom area of 100 mm^2 (10x10 mm). Then, the weight was placed on the excised tissues, stayed for 2 min and pulled at a speed of 0.1~2.0 mm/min to measure the maximum separating forces between the hydrated film and the tissues of the abdominal walls. INSTRON 4465 with a load cell of 250 g was used.

The dry adhesiveness, reattachment and wet adhesiveness were determined and the data were given in Table 3, below.

<TABLE 3> Maximum Adhesive Strength of Film Formulations

Nos. of Examples	Dry Adhesiveness(g)	Reattachment (%)	Wet Adhesiveness(g)
C. 1	21.8±1.5	55.2±3.8	15.1±0.3
C. 2	25.2±2.1	56.7±3.6	17.4±0.6
C. 3	25.6±1.6	36.3±2.5	8.7±1.2
C. 4	23.4±1.2	58.1±4.0	16.9±0.7

C. 5	29.4±2.0	42.9±2.7	11.2±0.4
1	26.8±1.5	89.1±4.5	19.0±0.5
2	27.7±2.3	81.2±3.1	17.5±0.9
3	24.9±1.7	88.4±3.9	17.9±0.8
4	24.8±2.1	79.4±4.7	18.2±1.1
5	25.3±1.4	81.6±3.2	16.7±0.9
6	25.7±1.7	74.4±2.9	14.8±0.6

In a dry state, as apparent from Table 3, the semi-IPN film formulations prepared according to the present invention showed similar initial adhesive strengths to those of the film formulations consisting solely of SA-Ca^{2+} or SCMC and a little lower initial adhesive strength than IPN structure films. As mentioned previously, since hydration was the most important factor in determining the initial adhesiveness, the films consisting of polysaccharides were not significantly different in the initial adhesiveness from one to another when they are hydrated to a certain level or higher. However, the semi-IPN film formulations showed exceptionally higher reattachment than the conventional ones. The reason for such higher reattachment was that, since the hydration of the film, the main factor of the initial tackiness, was achieved to some degree during their contact with body tissues, the adhesiveness of the formulations themselves to body tissues took the leading part in determining the reattachment. Namely, the semi-IPN structures of the present invention had excellent adherence to body tissues by virtue of the diffusion of the terminal carboxy groups of CMC, which do not participate

in crosslinking, while maintained their physical rigidity by crosslinks between alginate and calcium ions. The excellent reattachment gives a great contribution to the convenience upon operative or re-operation procedure.

Films prepared from SA, SCMC, or the simple mixture thereof, although anticipated highly in the adhesiveness to body tissues, showed poor reattachment because their physical rigidities are terminated owing to the dissolution which occurs as soon as they contact with water.

In a wet state, the most influence on the adhesive strength is the adhesiveness of the polymer itself to body tissues. Thus, the formulations consisting solely of SA, SCMC or SA-SCMC, which show adherence to body tissues owing to the diffusion of the terminal carboxy group, are relatively high in the adhesive strength in a wet state, rigid films of such formulations as SA-CA^{2+} and IPN, whose almost all terminal carboxy groups take part in the crosslinking with calcium ions, have no adhesiveness to body tissues, showing very poor adhesive strength in a wet state.

Compared with the other formulations, the semi-IPN formulations of the present invention had high adhesive strength in a wet state. This was also attributed to the same reason that while the crosslinked structure of alginate-calcium maintained the physical strength, CMC was responsible for the adhesiveness to body tissue.

With a view to investigating the effect of calcium ion

concentrations on the adhesiveness, the semi-IPN gel formulations of Examples 2 and 3 were subjected to the *ex vivo* adhesive strength test described above. The dry adhesiveness, reattachment and wet adhesiveness were measured and the data was given in Table 3.

The initial adhesiveness in a dry state, as mentioned earlier, is dependent greatly on the hydration of the formulations. Even when an amount of the calcium ions is changed, the hydration at initial stages is not significantly changed, so there are no great differences according to change of the calcium ion concentration. However, the reattachment and the wet adhesiveness are determined mainly by the adhesiveness to body tissue of CMC. Thus, as the concentration of calcium ions were increased, the adhesive properties become poor because some of CMC participated in the crosslinking.

In order to investigate the effect of the ratio between SA and SCMC on the adhesiveness, the semi-IPN gels of Examples 4, 5 and 6 were subjected to the *ex vivo* adhesiveness test.

No great changes were determined when the ratio between CMC and alginate was changed because the hydration had a critical influence on the dry adhesive strength at an early stage. However, as the ratio of CMC was reduced, the concentration of the terminal carboxy groups which played an important role in determining the adhesiveness was lowered, thus shows the poor reattachment and the poor adhesiveness in a wet state.

Experimental Example 5: Measurement of Strength and Elongation of Film Formulations

Thin films prepared from various formulations were cut into a dimension of 10x120 mm and strength and elongation were measured under the following conditions: sample gauge 50 mm and measuring speed 30~70 mm/min. To measure the physical properties of wet films, samples were inserted between two sheets of filter paper soaked in water and allowed to stand 2 min. The strength and elongation of the samples hydrated, were measured in the same manner. In this regard, INSTRON 4465 was available using a load cell of 100 kg for measuring the strength and elongation in a dry state and using a load cell of 250 g for measuring the strength and elongation in a wet state. The results were given in Table 4, below.

<TABLE 4> Strength and Elongation of Film Formulations

Nos. of Examples	Dry State		Wet State	
	Max. Strength (g)	Elongation (%)	Max. Strength(g)	Elongation (%)
C.1(SA)	4861±377	10.7±2.15	0.49±0.09	10.4±2.41
C.2(SCMC)	3854±153	5.64±1.78	8.25±1.64	60.4±4.78
C.5(SA-SCMC)	6214±256	12.2±1.96	6.81±1.85	47.3±4.56
C.6(IPN)	695±94	13.2±2.77	198±10.3	84.5±6.68
1(semi-IPN)	7039±231	16.9±1.24	72.4±5.37	68.8±5.63

As aforementioned, strength and elongation can be used as indices which indicate rigidity and flexibility of films,

respectively. In the present invention, an examination was made of the strength and elongation of the film in a dry state in order to establish a convenience standard on the first use and in a wet state in order to establish the readiness for a secondary operation.

As recognized from Table 4, there were no significant differences in strength in a dry state between the semi-IPN structure film and the simply mixed formulations, whereas the semi-IPN structure film was far superior to all of the rest, but the IPN formulation, in strength in a wet state. In contrast to the conventional formulations readily soluble to water, the semi-IPN structure film had a decreased solubility in water on account of the crosslinks between alginate and calcium ion. The IPN formulation was excellent in strength and elongation in a wet state, but very poor in strength in a dry state. Convenience in surgical operation was accomplished in the present invention which was not obtained in the IPN formulation.

Therefore, the high strength of the formulations in a wet state according to the present invention overcame the inconvenience of conventional polysaccharide film formulations.

Experimental Example 6: Animal Test for Antiadhesion Efficacy According to Formulation and Composition

6-1: Adhesion Forming Animal Model Test (Control)

After being etherized, female Sprague-Dawley rats with a body weight of 200~250 g had their central laparotomy. Their caeca were exposed from the abdominal cavity and rubbed with sand paper until blood spots appeared thereon. On the other hand, the epithelium at the left abdominal wall site was removed to form an injury of 1 cm x 1 cm. The caecum was rearranged near the injured site within the abdominal cavity, after which the abdominal wall was stitched with 4-0 silk sutures and the skin layer with 3-0 silk sutures. After 7 days, the rats were euthanized and underwent laparotomy to investigate the formation of adhesion (occurrence and severity). The index of the adhesion occurrence is the adhesion of the caecum with regard to the abdominal wall.

The following grade system was used to evaluate the severity of the adhesions.

0 grade: no adhesions occurred

1st grade: tiny avascular tissues adheres to the injured site, which can be removed easily with blunt instrumentslipids in the abdominal cavity somewhat adhered to the injured site.

2nd grade: the mesentery adhered to the injured site, forming a thread-like band, the adhesion can only be removed by sectioning.

3rd grade: vascular tissues were well developed and the caecum seriously adhered to the abdominal wall.

36 female SD rats were tested for the formation of adhesions, according to the method and the results were given as follows:

Grades	Numbers
0	1
1	4
2	6
3	25
TOTAL	36

From the data, the average adhesion score (A.S.) and the standard error of mean (S.E.M.) were 2.53 and 0.14 respectively as calculated according to the following formulas:

$$A.S. = \frac{\sum (Nos.\ of\ population\ by\ Grade \times Grade)}{Total\ Nos.\ of\ population}$$

$$S.E.M. = \frac{standard\ deviation}{\sqrt{Total\ Nos.\ of\ population}}$$

A.S. means the degree of the adhesion formed in an animal group. The A.S. value lies between 0 and 3. The higher the value, the more serious the adhesion and vice versa. S.E.M. means the difference between individual test animal groups. Smaller S.E.M values indicate smaller difference between groups.

6-2: Animal Test for Antiadhesion Efficacy According to

Formulations

Under the conditions described above, each of the gel and film formulations prepared in Example 1 and Comparative Examples 1 to 6 was applied to 36 SD rats to investigate their antiadhesion efficacy. After the rats were injured according to Experimental Example 6-1, films with a size of 4x5 cm^2 and 1.5x2 cm^2 were applied to the caecum and the abdominal wall, respectively. In case of gels, they were used at an amount of 2 ml. After one week, the rats were euthanized, followed by a careful observation of the formation and severity of adhesions. The results are given in Table 5, below.

<TABLE 5> Antiadhesion Effects According to Formulations

Formulations		A.S	S.E.M
Comparative Example 1(SA)	Gel	2.58	0.21
	Film	2.82	0.21
Comparative Example 2(SCMC)	Gel	1.67	0.31
	Film	1.88	0.21
C. 3(SA-Ca^{24})	Film	2.59	0.32
C. 4(SCMC-Al^{34})	Film	2.88	0.26
C. 5(SA-SCMC)	Gel	1.79	0.12
	Film	2.69	0.16
C. 6(IPN)	Film	2.66	0.32
Example 1(semi-IPN)	Gel	0.31	0.12
	Film	0.28	0.09

As apparent from the data of Table 5, the semi-IPN structure antiadhesion barriers of the present invention, in both gel and film formulations, exerted exceptionally more potent antiadhesion efficacy on the animal model than the conventional antiadhesion barriers based on the formulations consisting of SA, SCMC or a mixture thereof or on an IPN structure.

6-3: Animal Test for Antiadhesion Efficacy According to Ratios Between SA and SCMC

Films were prepared with various ratios of SA and SCMC in the same manner as in Example 1 and tested for antiadhesion performance in accordance with the indication of Experimental Example 6-1. The results were given in Table 6, below.

<TABLE 6> Antiadhesion Efficacy According to Ratios of Components.

Ratio	SA(%)	10	20	40	50	60	80	90
	SCMC(%)	90	80	60	50	40	20	10
Grades	0	26	28	22	17	13	11	10
	1	8	6	10	15	13	10	7
	2	1	2	3	2	6	8	7
	3	1	0	1	2	4	7	12
Total Numbers		36	36	36	36	36	36	36

A.S.	0.361	0.278	0.528	0.694	1.028	1.306	1.583
S.E.M	0.114	0.094	0.129	0.137	0.167	0.186	0.205

As recognized from Table 6, the semi-IPN structure antiadhesion barrier of the present invention showed excellent adhesion prevention effects over the whole range of ratios tested with high preference to a composition comprising 10~50 wt% of SA and 90~50 wt% of SCMC.

INDUSTRIAL APPLICABILITY

The antiadhesion barrier of the present invention is composed mainly water-soluble and carboxymethyl cellulose with the alginate crosslinked by calcium ions. The barrier has a semi-interpenetrating network structure as a result of the corsslinking of the alginate with the calcium ions. In addition, to being superb in reattachment and bioreadhesiveness, the antiadhesion barrier of the present invention shows high structural integrity maintenance and retains a certain degree or higher of strength even in a wet state. Also, it lacks blood anticoagulant effects, so that it can be applied for the surgery in which the surgical sites are relatively large or bleeding flows injury healing. Further, the antiadhesion barrier of the present invention is easy to handle and useful in surgery and secondary operative procedure.

Those skilled in the art will appreciate that the conceptions and specific embodiments disclosed in the foregoing description may be readily utilized as a basis for modifying or designing other embodiments for carrying out the same purposes of the present invention. Those skilled in the art will also appreciate that such equivalent embodiments do not depart from the spirit and scope of the invention as set forth in the appended claims.

WHAT IS CLAIMED IS:

1. An antiadhesion barrier, comprising a water-soluble alginate and a carboxymethyl cellulose as main components, said a water-soluble alginate being selectively crosslinked with calcium ions, wherein the antiadhesion barrier is useful to prevent formations of adhesions between body tissues during or after surgery.

2. The antiadhesion barrier of claim 1, wherein a weight ration of the alginate and the calcium ions ranges from 1:0.05 to 1:0.2.

3. The antiadhesion barrier of claim 1, wherein the alginate is with a range of 10~90 wt% and the carboxymethyl cellulose is with a range of 90~10 wt%.

4. The antiadhesion barrier of claim 3, wherein the alginate is with a range of 50~10 wt% and the carboxymethyl cellulose is within a range of 90~50 wt%.

5. The antiadhesion barrier of claim 1, wherein the antiadhesion barrier is a film

6. A method for preparing the antiadhesion barrier of claim 1, comprising the steps of:

1) dissolving a mixed powder of alginate and carboxymethyl cellulose in water to produce a solution;
2) adding a calcium ion solution to the solution while slowly stirring to give a gel solution;
3) molding the gel solution into a thin film; and
4) drying the film,

wherein the calcium ion solution has a weight ration of the alginate and the calcium ions ranging from 1:0.05 to 1: 0.2

7. A method for preparing the antiadhesion barrier of claim 1, comprising the steps of:

1) mixing an alginate solution and a carboxymethyl cellolose solution;
2) adding a calcium ion solution to the solution while slowly stirring to give a gel solution;
3) molding the gel solution into a thin film; and
4) drying the film,

wherein the calcium ion solution has a weight ration of the alginate and the calcium ions ranging from 1:0.05 to 1: 0.2

8. The antiadhesion barrier of claim 1, wherein the antiadhesion barrier additionally incorporates a drug.

9. The antiadhesion barrier of claim 1, wherein the

drug is selected from the group consisting of non-steroidal anti-inflammatory agents, anticoagulants, protein hydrolyzing agents, and growth hormone.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR00/00772

A. CLASSIFICATION OF SUBJECT MATTER

IPC7 A61K 9/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimun documentation searched (classification system followed by classification symbols)
A61K 9/00

Documentation searched other than minimun documentation to the extent that such documents are included in the fileds searched

Electronic data base consulted during the intertnational search (name of data base and, where practicable, search trerms used)
CA Online

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5266326 (PFIZER HOSPITAL PRODUCTS GROUP) 30. November 1993 (30. 11. 93) see the entire document.	1-9
A	EP 507604 (ETHICON) 07. October 1992 (07. 10. 92) see the entire document.	1-9

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevence
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevence; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevence; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents,such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/KR00/00772

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5266326	30. 11. 93	AU 671114 B2	15. 08. 96
		EP 648122 A1	19. 04. 95
		EP 648122 B1	20. 11. 96
		WO 9400134 A1	06. 01. 94
EP 507604 A2	07. 10. 92	AU 141592 A1	08. 10. 92
		JP 2702641 B2	21. 01. 98
		US 5532221	02. 07. 96